

## KINETIC MODELING OF ENZYMATIC HYDROLYSIS OF POPLAR WASTE BY WET OXIDATION PRETREATMENT

Shanshan Liu,<sup>a</sup> Guigan Fang,<sup>a,\*</sup> Qiang Wang,<sup>b</sup> Yongjun Deng,<sup>a</sup> and Shanming Han<sup>a</sup>

Kinetic modeling of enzymolysis of pulp fiber waste pretreated by a wet oxidation process was studied. First, a wet oxidation pretreatment of fiber waste was carried out. It was found that hemicellulose were removed efficiently, yielding material with a pentosan content of 4% (based on the o.d. biomass). A statistical kinetic model was developed based on the experimental results of the enzymatic hydrolysis of wet oxidation pretreated material. The model can be described as follows: RSY (reducing sugar yield) =  $12.48 \times C^{0.4261} \times (1 - e^{-0.2021t}) \times 100\%$ , an equation that can be used to predict the reducing sugar yield in an enzymolysis process. The RSY by enzymatic loading of 35FPU/g achieved almost the highest yield after 48 h, and there was no significant improvement with further extension of the enzymolysis time. The modeling was validated within the enzymatic loading range of 15 to 35FPU/g and provided a satisfactory interpretation of the experimental data.

*Keywords:* Enzymolysis; Cellulase; Reducing sugar; Poplar waste

*Contact information:* a: Institute of Chemical Industry of Forest Products, Chinese Academy of Forestry 210042, Nanjing, China.; b: State Key Lab of Pulp and Paper Engineering, South China University of Technology, Guangdong Public Laboratory of Paper Technology and Equipment, Guangzhou, China;

\* Corresponding author: pulpCAF@163.com

### INTRODUCTION

The pulp and paper industry, which converts lignocellulose into fiber-based products, has become a global industry and it is experiencing a rapid development, particularly in China (Zhang et al. 2007). During production in pulp mills, the raw material needs screening at first and it generates a large quantity of fiber waste (about 8%). Previously, the fiber waste was landfilled or burnt (Cavka et al. 2011). However, landfilled requires land, costing capital, and causing secondary pollution for soil, water and air (Zhao et al. 2005); burning could generate low heat values due to the considerable amounts of water. Considering the components of this waste (cellulose, hemicellulose, and lignin), it can be converted to ethanol instead of being landfilled or burnt. To date few studies have been devoted to ethanol production from pulp waste (Sjöde et al. 2007; Taherzadeh et al. 2008).

The conversion of lignocellulosic materials to ethanol generally proceeds as follows: pretreatment, hydrolysis of cellulose (hemicellulose) to monomer sugars, sugar fermentation, and recovery and purifying the ethanol (Fang et al. 2010). In contrast to the production of ethanol from sugar-rich or starch-rich materials, pretreatment is necessary due to its compact structure. The purpose of pretreatment is to remove lignin from

lignocellulose, reduce the crystallinity of cellulose, and enhance the porosity of the material, which can benefit the following enzymatic hydrolysis (Jafari et al. 2011).

Numerous pretreatment methods have been investigated for lignocellulose, including physical, chemical, and biological processes (Sun et al. 2002). Among the pretreatments, the wet oxidation pretreatment (WO) was shown to be a promising method to remove hemicellulose and lignin. With the presence of air and oxygen at relatively high temperature, two types of reactions occur, including a low-temperature hydrolytic reaction and high-temperature oxidative reaction (McGinnis et al. 1983). As literature reports, the combination of alkali and WO can reduce the formation of toxic furaldehydes and phenol aldehydes (Bjerre et al. 1996; Klinke et al. 2002).

Hydrolysis can be divided into chemical and enzymatical processes. Chemical hydrolysis involves the exposure of material to weak mineral acids at a specific condition to release sugar monomers. The drawback is the generation of substances (acetate) that are toxic to fermentation (Cheng et al. 2011). Enzymatic hydrolysis involves enzymatic reactions that convert cellulose into glucose and hemicellulose into pentoses and hexoses. The processes are catalyzed by cellulase and hemicellulase enzymes, and is the preferred step for cellulose saccharification, especially with the advances in enzyme technologies and when there is a priority to achieve low environmental impacts (Zhu et al. 2009).

This article deals with the kinetic modeling of enzymatic hydrolysis of pretreated lignocellulose material. The enzymatic loading of (15-35 FPU/g) and hydrolysis time of (2-72 h) were selected according to literature data and previous experience. The effect of WO pretreatment on lignocellulose and suitable conditions for enzymatic hydrolysis condition were established.

## **EXPERIMENTAL**

### **Material and Chemicals**

Poplar waste used in this work was collected from the peroxide mechanical pulping (P-RC APMP) screening stage of Shandong Chenming Paper Co., Ltd., China. The material was air dried at room temperature and stored in a polythene bag. The chemical components were as follows: cellulose content 47.28% (GB/T5515-1985), Klason lignin 25.63% (GB/T 2677.8-1993), ash content 3.63% (GB/T 2677.3-1993), ethanol-benzene extractives 1.1% (GB/T 2677.6-1993), pentosan content 22.61% (GB/T 2677.9-1994), and moisture content 11.63% (GB/T 2677.2-1993).

Cellulase L-10 used in this work was provided by KDN Biotech Group, Tsingtao, China. All chemicals were from commercial sources.

### **Wet Oxidation Pretreatment**

Wet oxidation pretreatment was carried out in oxygen pressure vessels of 2.5 L. The conditions were as follows: solid to liquid ratio 1:15, initial pH value 10 (adjusted by Na<sub>2</sub>CO<sub>3</sub>), oxygen pressure 12 bar, desired temperature 195°C, and duration of treatment 15 min. At the end of the extraction, the residual solid was separated by filtration, washed with distilled water, and then air dried at room temperature.

## Enzymatic Hydrolysis Process

A conical flask of 250 mL was selected for the enzymatic hydrolysis and placed in thermostatic cultivation. The concentration of solid for hydrolysis was 2%, diluted by 0.1 M acetate buffer (pH 4.8). The cellulose was treated with cellulase enzyme from 15 to 35 FPU/g. Hydrolysis time was varied from 2 to 72 h. After the desired time was reached, the hydrolyzate was separated and collected by filtration. The reducing sugar content in the hydrolyzate was detected by the DNS method and calculated by Eq. 1,

$$\text{RSY} = \frac{m_{\text{RS}}}{m_{\text{T}}} = \frac{nCV}{m_o(1-\omega)} \times 100\% \quad (1)$$

where RSY is the reducing sugar yield (%),  $m_{\text{RS}}$  is the content of reducing sugar (g),  $m_{\text{T}}$  is the substrate mass (g),  $m_o$  is the substrate weighted material (g),  $\omega$  is the moisture content (%),  $C$  is the concentration of reducing sugar ( $\text{g L}^{-1}$ ),  $V$  is the total volume of hydrolysate (L), and  $n$  is the dilution time.

## Analytical Methods

The amount of reducing sugar released from enzymatic hydrolysis was measured by the DNS method (Pala et al. 2001; Liu et al. 2008). The procedure was as follows: A certain volume of hydrolysate was added into a 25 mL tube, which contained 3 mL of DNS reagent. This was heated in the tube to the boiling point and kept at that temperature for 5 min. Next the tube was cooled in tap water. The absorbance at 540 nm was measured using a UV-vis spectrophotometer.

The calibration curve of glucose at different concentration was established as  $y=0.9476x-0.0634$  ( $R^2=0.9998$ ), where  $y$  is absorbance and  $x$  is glucose concentration (in mg/mL).

The cellulase activity was measured by filter paper enzyme activity (Mandels et al. 2009). The process was as follows: A strip of Whatman No. 1 filter paper ( $1 \times 6$  cm) was added into a 25 mL tube, with 1.5 mL enzyme solution containing 0.1 M acetate buffer. The pH value was kept at 4.8. Then the samples were incubated at 50°C for 1 h, after which the reducing sugar content was determined by the DNS method as described above.

The compositions of hydrolyzate from WO pretreatment were measured by HPLC (Agilent 100) using a Shodex sugar SP-ob10 column coupled with a refractive index detector, and degradation products were detected by an ultraviolet detector. The mobile phase was HPLC-grade water at a flow rate of 0.7 mL/min, with a column temperature of 80°C. The yield of sugars in the hydrolyzate was calculated on the basis of o.d. biomass. Before analysis, the hydrolyzate was hydrolyzed in  $\text{H}_2\text{SO}_4$  (2M) at 121°C for 1h (Zhu et al. 2010).

## RESULTS AND DISCUSSION

### Results of WO Pretreatment

Sixty grams (oven dry) of poplar waste were prepared for the WO pretreated in a digester, as described above. The composition of residual solid was determined by

chemical method, and HPLC was employed for measurement of the hydrolyzate. The results are listed in Table 1.

**Table 1.** Composition of Residual Solid and Hydrolyzate after WO Pretreatment

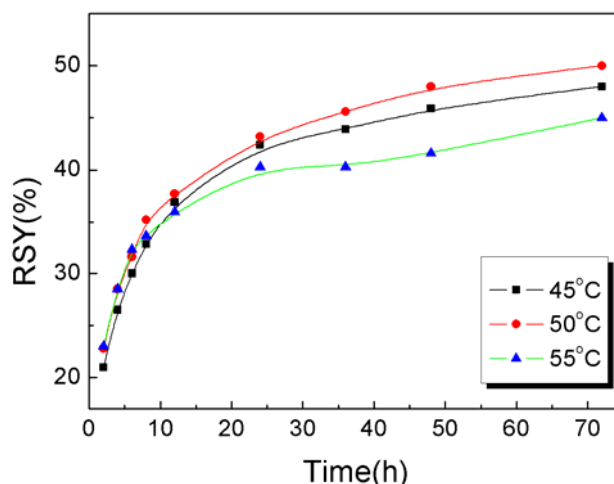
| Solid (% o.d. mass) |          |               | Hydrolyzate ( $10^{-2}$ % o.d. mass) |         |         |             |             |       |          |
|---------------------|----------|---------------|--------------------------------------|---------|---------|-------------|-------------|-------|----------|
| Cellulose           | Pentosan | Klason lignin | Xylose                               | Glucose | Xylitol | Formic acid | Acetic acid | HMF   | Furfural |
| 60.83               | 4.00     | 33.19         | 5.13                                 | 1.20    | 2.1     | 2.78        | 3.57        | 0.568 | 2.08     |

As can be seen from Table 1, hemicellulose remaining in the residual solid dropped to 4% (present as pentosan), and cellulose comprised more than 60%. In the solution of pretreated WO, the xylose was  $5.13 \times 10^{-2}$  %, which is higher than hot-water treatment of  $2.17 \times 10^{-2}$  % and SPORL (1.9) of  $3.18 \times 10^{-2}$  % carried out on lodgepole pine (Zhu et al. 2010), which indicate that the WO pretreatment can remove hemicellulose efficiently, making it suitable for enzymolysis.

### Optimization the Temperature for Enzymatic Hydrolysis

It is well known that cellulase is a kind of protein. Thus it is understandable that although the reaction between enzyme and solid could be accelerated in the proper range of temperature, it also can be denatured and inactivated when temperatures beyond the range of tolerance are employed (Li et al. 2007). Therefore, temperature is a key parameter that should be considered.

Two grams of solid were weighted into a beaker at a loading of 25 FPU/g cellulase, and then the beaker was placed in the thermostatic cultivation. The desired temperature was varied from 45°C to 55°C. The reducing sugar content in the solution was determined at different times. Results are shown in Fig. 1.



**Fig. 1.** The reducing sugar yield v.s. temperature

As presented in Fig. 1, the RSY for three different temperatures was similarly from the start until 10 h, and then distinctly different behavior was observed. The

temperature of 50°C showed better RSY than either 45°C or 55°C; therefore the optimal temperature for the cellulase should be 50°C.

### Kinetics of Enzymatic Hydrolysis Using Cellulase

A series of cellulose hydrolysis tests was carried out to optimize the reducing sugar yield and model the kinetics. The experimental process was conducted as described above, and a temperature of 50°C was fixed for all experiments. The cellulose was treated with cellulase enzyme from 15 to 35 FPU/g, and the time was varied from 2 to 72 h. The reducing sugar content in hydrolyzate was measured as described earlier.

As shown in Fig. 2, RSY in hydrolyzate increased steadily as hydrolysis proceeded under the conditions studied. When the applied enzymatic loading increased from 15 FPU/g to 35 FPU/g, the RSY increased by more than 54%, suggesting that the enzymatic loading has a profound impact on the extent of RSY. At the end of enzymatic hydrolysis, the RSY typically ranged from 44.77% to 65.17%.

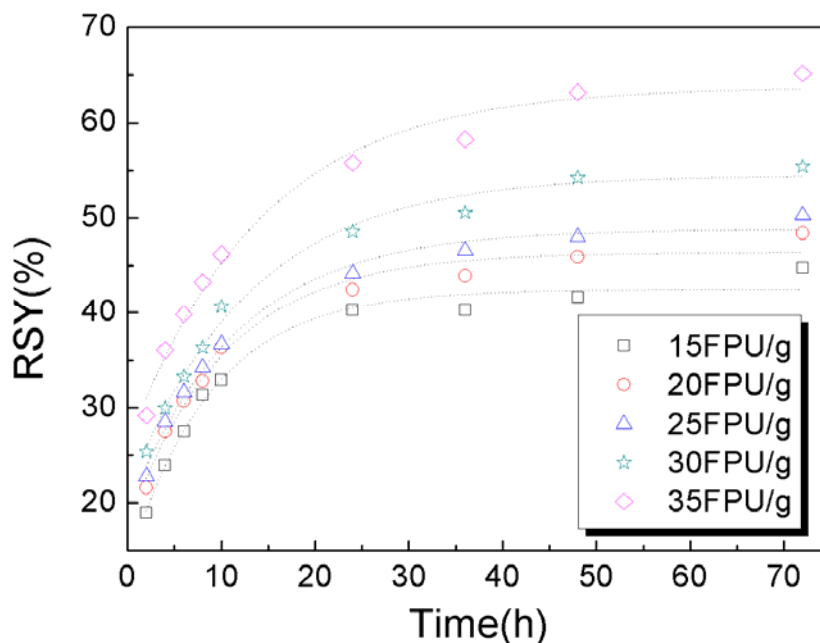
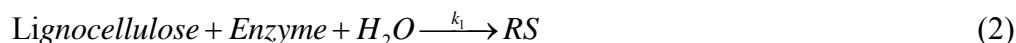


Fig. 2. Effect of reaction time and enzyme loading on the RSY

It is clear from the results that the enzymolysis process can be divided into two distinct phases. The initial fast phase occurred in the range 2 to 24 h. This was followed by a slow phase, during the period from 24 to 72 h. RSY during the first phase can be increased by about 100%, followed by about 20% increment during the slow phase.

### Kinetic Model Development

Cellulose hydrolysis is a type of chemical reaction based on molecular motions, including adsorption of enzymatic molecule onto substrate molecules, formation of chemical complexes, and ultimately dissociation (Um et al. 2010). The following apparent kinetic mechanism is proposed to simplify the reaction process (Tu et al. 2008),



where *Lignocellulose* is the enzymolysis substrate, *Enzyme* is cellulase, and RS is reducing sugar.

From Eq. 2 the kinetic equation of RS producing for enzymatic hydrolysis can be derived as follows,

$$v_{\text{RS}} = \frac{dm_{\text{RS}}}{dt} = k_1 m_T^n C^m \quad (3)$$

where  $v_{\text{RS}}$  is the rate of enzymatic hydrolysis,  $m_{\text{RS}}$  is the reducing sugar content in the liquor (g),  $m_T$  is the total substrate mass (g),  $C$  is the enzyme charge during enzymatic hydrolysis (FPU/g),  $K_1$  is the rate constant of the reaction ( $K_1=f(1/T)$ ,  $\text{g.L}^{-1} \text{min}^{-1}$ ),  $t$  is reaction time, and  $n$  and  $m$  are the power of each parameter.

During enzymatic hydrolysis, the concentration of enzyme in the hydrolysate is hardly changed. Thus the expression can be simplified as  $k_2 = k_1 C^m$ , and Eq. 3 can be simplified as Eq. 4:

$$\frac{dm_{\text{RS}}}{dt} = k_2 m^n = k_2 (m_T - m_{\text{RS}})^n \quad (4)$$

Equation 5 can be obtained by integrating Eq. 4:

$$\int_0^m \frac{dm_{\text{RS}}}{(m_T - m_{\text{RS}})^n} = \int_0^t k_2 dt \quad (5)$$

Enzymatic hydrolysis can be assumed to be a first-order reaction (Garrote et al. 2001). Thus, Eq. 6 can be derived from Eq. 5:

$$m_{\text{RS}} = m_T (1 - e^{-k_2 t}) \quad (6)$$

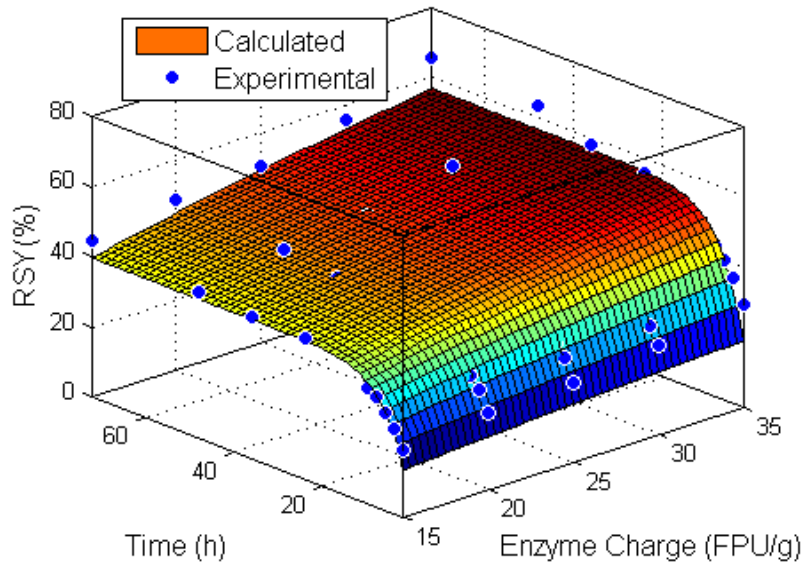
Additionally, the enzymatic loading has a major effect on RSY, as shown in Fig. 2. A higher enzymatic loading, in the range of 15FPU/g to 35FPU/g, resulted in more reducing sugar. Therefore, a concentration term of enzyme should be added to Eq. 6, and the rate of enzymatic hydrolysis can be described from Eq.1 and Eq. 6 as follows,

$$\text{RSY} = K \times C^a \times \frac{m_{\text{RS}}}{m_T} \times 100\% \quad (7)$$

where  $C$  is the enzymatic loading, and “ $a$ ” represents the exponent of  $C$ . Substituting Eq. 6 into Eq. 7, the following expression is obtained:

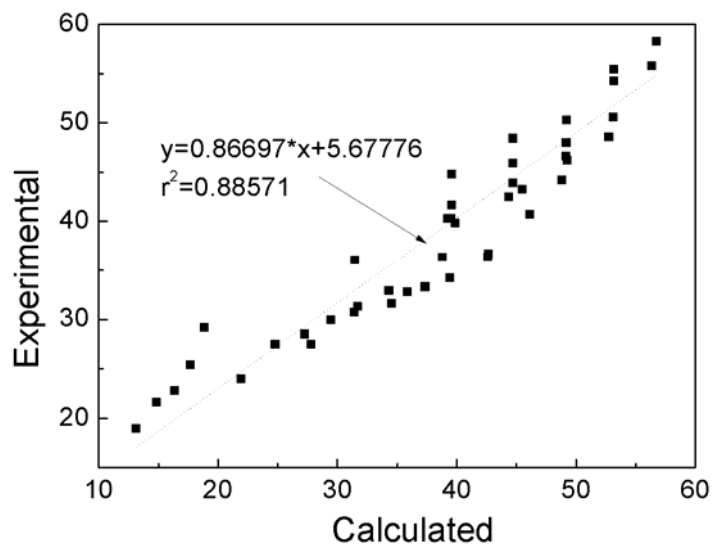
$$\text{RSY} = K \times C^a \times (1 - e^{-k_2 t}) \times 100\% \quad (8)$$

Using the experimental data, the least-square estimated of the parameters in Eq. 8 were estimated using nonlinear procedure of curve fitting toolbox in Matlab (R2010b, The MathWorks, Inc.). The fitting result is shown in Fig. 3.



**Fig. 3.** RSY as a function of reaction time and concentration; fitting results by Matlab

In the enzymatic hydrolysis process, the RSY was increased with elevated enzymatic loading and prolonged hydrolysis time, according to the experimental conditions. As shown in Fig. 3, the model based on Eq. 8 fit the measured data well. The parameters of  $K$ ,  $a$ , and  $k_2$  were 12.48, 0.4261, and 0.2021, respectively, and R-square for this estimation was 0.8644.



**Fig. 4.** Measured and model predicted RSY in the enzymatic hydrolysis

## Modeling Application

As shown in Fig. 4, a linear regression was applied to estimate the parameters of the mathematical model based on Eq. 8. The coefficient of determination 0.8857 was calculated. Therefore, this kinetic model derived experimentally can be used to predict the RS producing of poplar waste in the enzymatic hydrolysis within a range of 15FPU/g to 35FPU/g of cellulase loading. The good linear correlation between the model prediction and 45 independent experimental results supports the validity of the model, providing evidence of its suitability for application. With this model, the RS content in the hydrolyzate can be predicted from the enzymatic hydrolysis condition.

## CONCLUSIONS

Wet oxidation pretreatment is a promising process to remove about 90% of the hemicellulose present in pulp fiber waste. A new approach to quantitative modeling of reducing sugar yield in the enzymolysis process of fiber waste pretreated by wet oxidation was developed and tested. A kinetic model of reducing sugar producing by cellulase was proposed and justified as follows:  $RSY=12.48 \times C^{0.4261} \times (1-e^{-0.2021t}) \times 100\%$ . The kinetic coefficients of the modeling were calculated with an optimisation algorithm, achieving a close fit to the experimental data.

## ACKNOWLEDGEMENTS

The authors acknowledge the support of "Forestry industry research special funds for public welfare projects (development of enzymatic synthesis of bio-based fuel)"

## REFERENCES CITED

- Bjerre, A.-B., Olesen, A. B., Fernqvist, T., Plöger, A., and Skammelsen Schmidt, A. (1996). "Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose," *Biotechnology and Bioengineering* 49(5), 568-577.
- Cavka, A., Alriksson, B., Rose, S. H., and Jönsson, L. J. (2011). "Biorefining of wood: Combined production of ethanol and xylanase from waste fiber sludge," *Journal of Industrial Microbiology & Biotechnology* 38(8), 891-899.
- Cheng, J. J., and Timilsina, G. R. (2011). "Status and barriers of advanced biofuel technologies: A review," *Renewable Energy* 36(12), 3541-3549.
- Fang, X., Shen, Y., Zhao, J., Bao, X., and Qu, Y. (2010). "Status and prospect of lignocellulosic bioethanol production in China," *Bioresour. Tech.* 101(13), 4814-4819.
- Garrote, G., Domínguez, H., and Parajó, J. C. (2001). "Kinetic modelling of corncob autohydrolysis," *Biotechnology Letters* 36, 571-578.
- Jafari, V., Labafzadeh, S. R., Jeihanipour, A., Karimi, K., and Taherzadeh, M. J. (2011). "Construction and demolition lignocellulosic wastes to bioethanol," *Renewable*



*Energy* 36(11), 2771-2775.

- Klinke, H. B., Ahring, B. K., Schmidt, A. S., and Thomsen, A. B. (2002). "Characterization of degradation products from alkaline wet oxidation of wheat straw," *Bioresource Technology* 82(1), 15-26.
- Li, W., and Wu, Z. S. (2007). "Preliminary study on the conditions for enzymatic hydrolysis of wood," *Renewable Energy Resources* 25(2), 41-43.
- Liu, N., Qin, M. H., Wang, Y. Q. and Wang, Z. (2008). "Bioconversion of waste paper to fermentable sugar," *Proceeding of International Conference on Pulping, Papermaking and Biotechnology* 248-252.
- Mandels, M., Andreotti, R., and Roche, C. (2009). "Measurement of saccharifying cellulase," *Biotechnology for Biofuels* 2, 21.
- McGinnis, G. D., Wilson, W. W., and Mullen, C. I. M. E. (1983). "Biomass pretreatment with water and high-pressure oxygen. The wet-oxidation process," *Industrial & Engineering Chemistry Product Research and Development* 22(2), 352-357.
- Pala, H., Lemos, M. A., Mota, M. and Gama, F. M. (2001). "Enzymatic upgrade of old paperboard containers," *Enzyme and Microbial Technology* 29(4-5), 274-279.
- Sjöde, A., Alriksson, B., Jönsson, L. J., and Nilvebrant, N.-O. (2007). "The potential in bioethanol production from waste fiber sludges in pulp mill-based biorefineries," *Applied Biochemistry and Biotechnology* 136-140, 327-337.
- Sun, Y., and Cheng, J. Y. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technology* 83(1), 1-11.
- Taherzadeh, M. J., and Karimi, K. (2008). "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *International Journal of Molecular Sciences* 9(9), 1621-1651.
- Tu, Q. L., Fu, S. Y., Zhan, H. Y., Chai, X. S., and Lucia, L. A. (2008). "Kinetic modeling of formic acid pulping of bagasse," *J. Agricultural Food Chemistry* 56(9), 3097-3101.
- Um, B. H., and van Walsum, G. P. (2010). "Evaluation of enzyme mixtures in releasing fermentable sugars from pre-pulping extracts of mixed northeast hardwoods," *Applied Biochemistry and Biotechnology* 161(1-8), 432-447.
- Zhang, D. C., J. Hsieh, Chai X. S., and Ragauskas, A. J. (2007). "Experimental and modeling of carbonate formation in the effluent of oxygen delignification," *AIChE Journal* 53(3), 669-677.
- Zhao, C. S., Sun, X., Chen, X. P., and Gu, L. F. (2005). "Investigation on co-combustion characteristic of paper mill sludge, residue and coal in circulating fluidized bed," *Journal of Southeast University (Natural Science Edition)* 35(1), 95-99.
- Zhu, J., Wang, G., Pan, X. J., and Gleisner, R. (2009). "Specific surface to evaluate the efficiencies of milling and pretreatment of wood for enzymatic saccharification," *Chemical Engineering Science* 64(3), 474-485.
- Zhu, W., Zhu, J. Y., Gleisner, R., and Pan, X. J. (2010). "On energy consumption for size-reduction and yields from subsequent enzymatic saccharification of pretreated lodgepole pine," *Bioresource Technology* 101(8), 2782-2792.

Article submitted: June 19, 2011; Peer review completed: August 10, 2011; Revised version received: August 31, 2011; Accepted: Sept. 3, 2011; Published: Sept. 7, 2011.